A

UTILITY PATENT APPLICATION
UNDER 37 CFR 1.53(b)

HONORABLE COMMISSIONER OF
PATENTS AND TRADEMARKS
Washington D.C. 20231

Case Docket No. 114205.1200

Sir:

Transmitted herewith for filing is the patent application of:

INVENTOR: Denise R. COOPER and Niketa A. PATEL
FOR: GLUCOSE REGULATED mRNA INSTABILITY ELEMENT

Enclosed are:

[X] 124 pages of specification, claims, abstract
[] Declaration & Power of Attorney
[ ] Priority Claimed
[ ] Certified copy of ____________
[X] 46 formal drawings
[ ] An assignment of the invention to ____________ and the assignment recordation fee
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[ ] Information Disclosure Statement, Form PTO-1449
[ ] Copies of IDS Citations
[ ]

The filing fee has been calculated as shown below:

(1) FOR	(2) NO. FILED	(3) NO. EXTRA	(4) RATE	(5) AMOUNT
TOTAL CLAIMS	25	-20	5	x $18.00 = $90.00
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Total of above calculations		=		$928.00
[] Assignment & Recording Fee				$.00

Docket No.: 114205.1200

TOTAL FEE $928.00

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[] Any patent application processing fees under 37 CFR 1.17.
[] Any filing fees under 37 CFR 1.16 for presentation of extra claims.

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Respectfully submitted,

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November 8, 1999
DC: #132851 v1 (2%$B01!.WPD)

FAX

TRANSMITTAL

NOV 8 1999

LRR

To: Gianna Arnold
Pepper Hamilton LLP

Fax: 202 220-1665

From: Wendy Davis

Date: November 8, 1999

Pages: 15, including cover

Re: **NEW APPLICATION**
USF Ref No. 99A010
Glucose regulated mRNA...

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99-11-08A11:26 RCVD

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✓ 99A010 CC KGP-3/16/99

APPENDIX 11

CONFIDENTIAL

INVENTION DISCLOSURE FORM
DIVISION OF PATENTS AND LICENSING
UNIVERSITY OF SOUTH FLORIDA
FAO 126

DATE: 3/11/1999

DISCLOSURE NO.: 99A010

INVENTOR SUBMITTING DISCLOSURE: Denise R. Cooper and Niketa A. Patel

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SIGNATURE: Denise R. Cooper Niketa Patel

TITLE OF INVENTION: Glucose regulated mRNA instability element.

1. DIRECTIONS:

This form is to be completed and submitted to the Division of Patents and Licensing by any Researcher who believes he or she has developed a new invention. The purpose of this form is to permit the Divison of Patents and Licensing to determine whether any legal protection for the invention will be sought. **HENCE IT IS IMPORTANT THAT ALL QUESTIONS BE ANSWERED AS ACCURATELY AS POSSIBLE.**

2. THE INVENTION

A. What is the problem this invention addresses?

See attached document

B. In the space provided, please briefly describe and explain your invention in the form of an abstract. If the space provided is not sufficient, kindly attach the abstract to this Disclosure Form.

See attached document

***Title:* Glucose-regulated mRNA instability element**

2A. What is the problem this invention addresses?

Regulatable vectors for gene expression are often not reliable since they can be "leaky". They usually consist of systems where the gene of interest is cloned downstream of a minimal viral promoter fused to copies of the tetracycline operator. The promoters used with these repressor sequences are, however, not totally repressed in many cells. By engineering a construct that is also regulated at the post-transcriptional level, full repression of such a promoter could be achieved in a wider variety of cells. We propose that by inserting instability sequences down-stream of an inserted cDNA, genes can be further regulated using high extracellular concentrations of glucose or non-metabolized analogues. Both Tet and Retroviral Tet Systems (Clontech) show promise for regulating transcription of genes in cells. The ecdysone-inducible expression system derived from *Drosophila* is also commercially available (Invitrogen) and may have even lower basal activity in mammalian cells. Basal activity of the tetO.HCMC IE promoter is highly variable when tested in several cell lines. Although the promoter was repressed in HeLa and PC12 cells, basal levels were 10-30 fold higher in BHK cells. This high basal activity limits the use of the tetracycline-repressed promoter. If a construct could be engineered that was also regulated at the post-transcriptional level, full repression of such a promoter could be achieved in a wider variety of cells. There are several inducible promoters available, however, no one has developed a system to regulate gene expression at the post-transcriptional level. The post-transcriptional regulation of mRNA destabilization of a gene is another possibility for designing regulatable gene expression constructs. If the cDNA can be destabilized by a non-toxic nutrient or analogue, then expression can be stabilized when the analogue is withdrawn.

2B: The invention:

The *cis*-acting elements destabilizing mRNA in response to high extracellular glucose were identified by several criteria in the exon encoding the C-terminal 52 amino acids for PKCßII and are shown below. The PKCßII-specific exon is inserted into mature mRNA via alternative splicing of pre-mRNA. The elements inserted may form stem-loop structures providing the secondary structure recognized by destabilizing endonuclease-protein complexes that break A-T bonds (1).

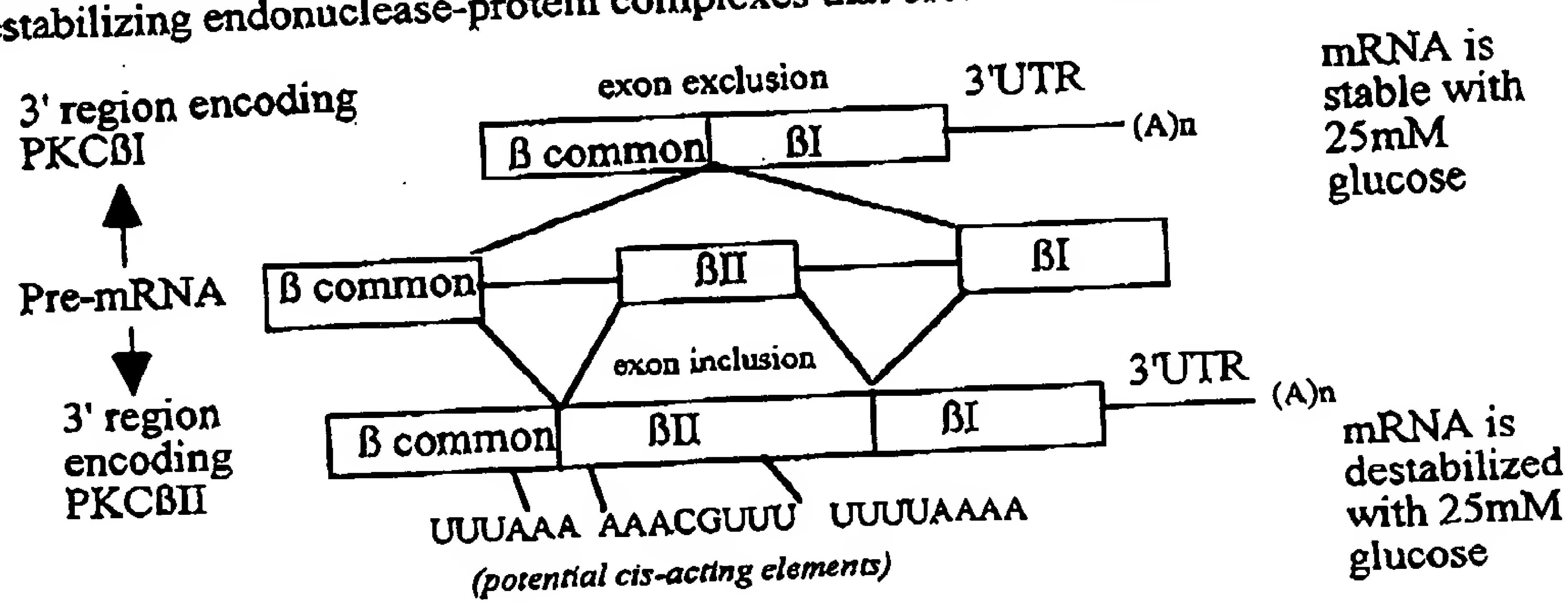


FIGURE 1: Diagram of 3' exons encoding PKCßI and PKCßII via exon inclusion/exclusion and regions of PKCßII exon involved in destabilization of mRNA.

The PKCß gene encodes two isoforms of the serine/threonine kinase that differ by their C-terminal fifty to fifty-two amino acids. If the exon for PKCßII is included in the mature mRNA transcript, PKCßII protein is encoded, the PKCßI exon is not translated due to a stop codon within the exon. If the PKCßII exon is excluded, PKCßI protein is encoded. The 3' untranslated region (UTR) of PKCßI is identical for both PKCßI and PKCßII mRNA. The mRNA for PKCßII is rapidly destabilized in the presence of high extracellular glucose (25 mM) in a number of cells. Like transcription, RNA processing, and translation, mRNA decay is a precise process dependent on specific *cis*-acting sequences and *trans*-acting factors. There are specific pathways triggering mRNA decay including poly (A) shortening, arrest of translation, and endonucleolytic cleavage. We have found that high extracellular glucose concentrations destabilize PKCßII mRNA in a rapid manner, and a nuclease activity in the cytoplasm is involved.

Analysis of the PKCßII exon sequence reveals multiple potential *cis*-acting elements that may be involved in the destabilization of the PKCßII sequence. These elements may form stem-loop structures that are recognized by putative carbohydrate response-acting factors to target the sequence for decay by cytosolic endonucleases (1).

The sequence could be placed down-stream of the cDNA insert to be expressed in cells, and high extracellular glucose or an analog could be maintained in the media to keep the mRNA of interest down-regulated. When expression of the gene is desired, lowering cell glucose levels to normal (5.5 mM) levels would allow for expression of the target mRNA. Conversely, a gene could be rapidly down-regulated by elevating high extracellular glucose. If the destabilization signal sequence were cloned into a plasmid also carrying the tetracycline repressor, it could fine-tune the system to keep basal levels of the gene low. Glucose or a non-metabolizable analogue provides an inexpensive, non-toxic alternative for regulating gene expression as opposed to expensive and potentially toxic antibiotics.

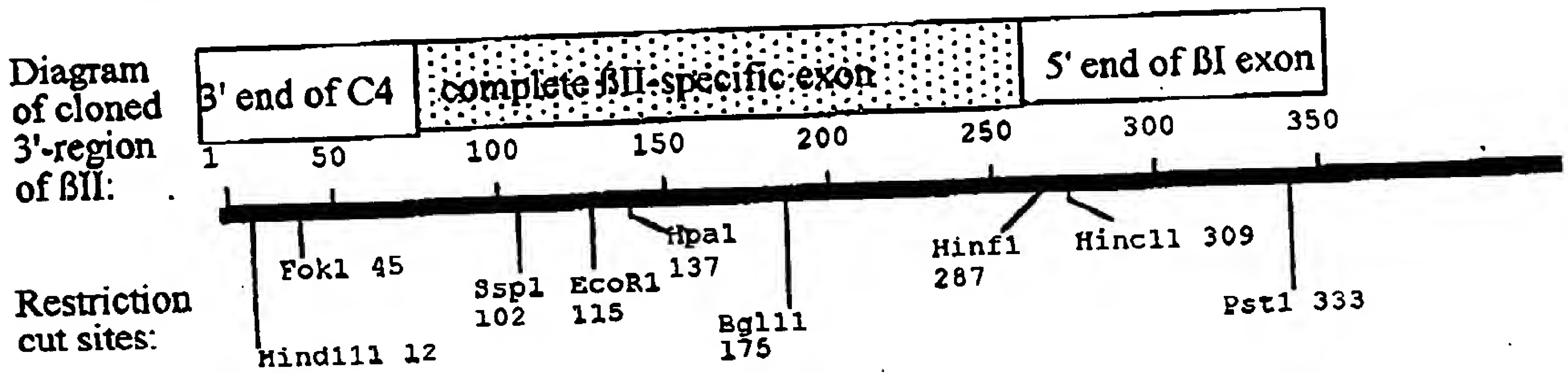
2C. Detailed description of the invention: The ßII-exon should destabilize a cDNA insert in the presence of high extracellular glucose. For our initial sutdies, the pßglobin (pßG) vector (obtained from Norman P. Curthoys, Colorado State University) was used (2). The chimeric pßG-ßII plasmid was constructed by inserting the PKCßII exon and flanking regions as shown below into the vector at a multicloning site. The vector was created by inserting the PvuII-Bgl II fragment of pSVß10, the portions of the first three exons and the two introns of the rabbit ß-globin gene, into the HindIII site of the pRc/RSV vector. The ß-globin genomic sequence extends from 9 bp upstream of the transcription initiation site to the translation stop codon. pßG contains a strong viral promoter derived from the long terminal repeat of the Rouse sarcoma virus followed by genomic DNA containing the transcriptional start site, the 3' nontranslated region, the full coding sequence and two introns of the rabbit ß-globin gene, a MCS with four restriction sites, and the 3'nontranslated region and polyadenylation site of the bovine growth hormone gene. Other chimeric constructs will also be tested such as CAT and luciferase to validate the effect of the ßII exon (3). The minimal region of the sequence will be used in tetracycline repressor construct systems (4). The chimeric reporter constructs are tested in a number of cell types. We have found that glucose destabilizes PKCßII mRNA in L6 rat skeletal muscle cells, rat aortic vascular smooth muscle cells, and MCF-7 breast cancer cells. Cells such as NIH-3T3 fibroblasts, HeLa cells, PC12 cells, and other tumorigenic and normal cell lines will also be tested for the ability of glucose to destabilize the chimeric construct.

To further define the minimal boundaries of the *cis*-elements, a portion of the 3' region of the the PKCßII mRNA as diagrammed above was obtained as a 404 bp insert containing the entire 216 bp ßII exon and 3' and 5' sequences from the flanking common and ßI exon (4). This insert will be restricted by deletion mutagenesis to further limit the amount of mRNA necessary for destabilizing genes of interest.

The destabilization of the PKCβII mRNA by high extracellular glucose is specific for PKCβII mRNA, PKCβI mRNA is not destabilized (4). To determine whether glucose or its metabolites destabilized the mRNA, two glucose analogues were tested. 3-O-methylglucose is taken up by cells but not phosphorylated by hexokinase. 2-Deoxyglucose is taken up by cells and phosphorylated but not further metabolized. Both analogues destabilized PKCβII mRNA. Thus, the further metabolism of glucose to glucose-6-phosphate is not required for this effect. This offers another means of regulating mRNA stability of the chimeric construct using glucose analogues.

2D. Drawing of the insert.

Figure 2: Restriction map of 3' region of PKCßII exon with portions of 3'- and 5'-flanking exons to be cloned downstream of the cDNA of interest.



References:
1. Patel, N. A., Chalfant, C.E., Yamamoto, M., Watson, J.E., Eichler, D.C., Cooper, D.R. Acute hyperglycemia regulates transcription and post-transcriptional stability of PKCßII mRNA in vascular smooth muscle cells. FASEB 13, No. 1: 103-113.
2. Hansen, W.R., Barsic-Tress, N., Taylor, L., Curthoys, N.P. The 3'-nontranslated region of rat renal glutaminase mRNA contains a pH-responsive stability element. Am. J. Physiol. 271 (Renal Fluid Electrolyte Physiol. 40): F126-F131, 1996.
3. Amara, F.M., Sun, J., Wright J.A. Defining a novel cis-element in the 3'untranslated region of mammalian ribonucleotide reductase component R2 mRNA. J. Biol. Chem. 271: 20126-20131, 1996.
4. Ackland-Berglund, C.E., Leib, D.A., Efficacy of tetracycline-controlled gene expression is influenced by cell type. Biotechniques 18: 196-200, 1995.

C. On a separate page(s), please attach a detailed description of the invention. Such a description must contain sufficient detail of the invention so that one skilled in the same discipline as yourself could reproduce the invention. If a manuscript explaining the invention has been prepared for submission to a journal, please attach a copy to this Disclosure form.

D. Have any drawings, photographs, graphs or data been prepared which pertain to the Invention?

Yes ____X__
No _______

If the answer to is yes, please attach those materials along with an explanation of what they demonstrate to this Disclosure Form.

E. Has a working model or prototype of the invention been made?

Yes _X_____
No _______

3. FINANCIAL SUPPORT

A. Was this invention discovered during the conducted research that was being performed for an outside sponsor under a research grant or contract?

Yes _X_____
No _______

B. If the answer to 3A is yes, please provide:

(1) the name of the outside agency (if the Federal Government, please state which department)

___**American Heart Association, Florida Affiliate**

(2) full address of sponsor, including name and telephone number of contact:

Research Administration

9900 Ninth Street N rth
St. Petersburg, FL 33716-3801

(3) the grant or contract number:

9810139FL

(4) the USF grant specialist who helped you obtain the grant:

Meg Damato, Sponsored Research

NOTE: If the research was conducted under research grants from more than one outside sponsor, on a separate piece of paper, please answer question 3B for each outside sponsor.

4. PUBLICATIONS

A. Has the invention been published?

Yes ______
No __X____

B. If the answer to 4A is yes, please provide the complete citation for the publication including the date of publication:

C. Do you intend to publish the invention?

Yes __X____
No ______

D. If the answer to 4C is yes, please provide the date on which the invention is to be published.

Manuscript in preparation.

E. Have you disclosed, or do you intend to disclose the invention to a scientific or professional association gathering in the form of either an oral presentation or a poster presentation?

Yes ____X____
No ______

Please circle the type of presentation: POSTER ORAL

F. If the answer to 4E is yes, please provide:

(1) the name of the scientific or professional organization:

Keystone Symposia

(2) the location of the gathering:

Sante Fe, New Mexico

(3) the date of the gathering:

Feb 23-28, 1999

G. Has the invention been publicly disclosed in any other manner?

Yes ___X___
No _______

H. If the answer to 4G is yes, please give the date on which the public disclosure was made and a detailed description of the circumstances under which the public disclosure was made:

Dissertation of Niketa A. Patel on November 6th 1998.

5. **PRIOR ART**

A. Are you familiar with any publications which disclose an invention similar to the invention disclosed in this Invention Disclosure Form?

Yes _______
No ___X___

B. If the answer to 5A is yes, please provide for each publication:

(1) the full title and citation of the publication:

(1) a brief explanation as to why the invention disclosed in this Invention Disclosure Form is an improvement over the invention disclosed in the publication:

(If more space is needed, please use a separate piece of paper.)

6. INVENTOR(S)

A. Please state the number of inventors of the Invention disclosed herein:

2

B. For each inventor, please state:

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Date: March 11/99

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Signature: Niketa Patel

Date: 3/12/99

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(9) Citizenship:

Signature:

Date:

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(9) Citizenship:

Signature:

Date:

NOTE: **If there are more than four inventors, please attach additional sheets for each inventor n t named here. If the inventions are described in a publication which identifies authors ther than the inventors, please describe the contributions made by the non-inventors.**

DISCLOSURE KEY DA[illegible] FOR DEALS ENTRY

Tech ID (next no. in sequence)	99A010			
Marketing Status (chosen from drop down list				
Manager of Disclosure				
Date on Disclosure	3/11/99			
Date Disclosure received by DPL				
Publication/Presentation Date(s)				
Inventors				
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Citizenship				
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% Revenue to be rcvd (from executed RAA)				
Interest of Technology (medical/industrial/etc.)				
Funding Information				
Funding Institution				
Grant No. of FI				
Grant Period				
FI Grant Manager/Phone No.				
USF Grant Account No. (from DSP Grant Specialist)				
Law Firm (if/when assigned and sent)				
Law Firm Reference No.				